

AMENDMENTS TO THE CLAIMS

1-70 (Cancelled)

71. (New) A method of rapidly introducing an exogenous molecule into a live population of cells, comprising the steps of:

providing a live population of cells in the presence of said exogenous molecule;

providing an energy source configured to deliver a beam of radiation;

positioning a container holding said live population of cells within the focus of said beam of radiation; and

contacting one or more cells of said live population of cells with said beam of radiation, wherein said beam of radiation has a diameter of at least 2 μm at the point of contact with said one or more cells;

wherein one or more cells of said live population of cells is transiently permeabilized to allow said exogenous molecule to enter.

72. (New) The method of Claim 71, wherein said diameter of said beam of radiation is at least 5, 7, 10, 15, 20, 25, or 30 μm at said point of contact with said live population of cells.

73. (New) The method of Claims 71 or 72, wherein said beam of radiation delivers at most 2 $\mu\text{J}/\mu\text{m}^2$.

74. (New) The method of Claim 71 or 72, wherein said beam of radiation delivers at most 1.5, 1, 0.7, 0.5, 0.3, 0.2, 0.1, 0.05, 0.02, 0.01 or 0.005 $\mu\text{J}/\mu\text{m}^2$.

75. (New) The method of Claims 71, wherein said beam of radiation is delivered through a lens with a numerical aperture of at most 0.5.

76. (New) The method of Claim 75, wherein said lens has a numerical aperture of at most 0.4, 0.3, or 0.25.

77. (New) The method of Claims 71 or 72, wherein positioning said container holding said live population of cells within said focus of said beam of radiation comprises:

illuminating a field-of-view containing said live population of cells;

analyzing an image of said live population of cells within said field-of-view; and

adjusting the distance between said population of cells and said lens.

78. (New) The method of Claim 75, wherein said lens has an F-theta flat-field correction.

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79. (New) The method of Claim 76, wherein said lens has an F-theta flat-field correction.

80. (New) The method of Claim 75, wherein said lens comprises a working distance of at least 5, 7, or 10 mm.

81. (New) The method of Claim 76, wherein said lens comprises a working distance of at least 5, 7, or 10 mm.

82. (New) The method of Claims 71 or 72, wherein the viability of the transiently permeabilized cells is more than 50%, 60%, 70%, 80%, 90%, 95%, or 98% after the method is performed.

83. (New) The method of Claim 71, wherein said exogenous molecule comprises a nucleic acid, a polypeptide, a carbohydrate, a lipid or a small molecule.

84. (New) The method of Claim 71, wherein said live population of cells comprises a prokaryotic cell.

85. (New) The method of Claim 71, wherein said live population of cells comprises a eukaryotic cell.

86. (New) The method of Claim 71, wherein said live population of cells comprises one cell selected from the group consisting of an animal cell, a plant cell, a yeast cell, a human cell and a non-human primate cell.

87. (New) The method of Claim 71, wherein the molecular weight of said exogenous molecule is greater than 0.1, 0.3, 1, 3, 10, 30, 70, 100 or 200 kiloDaltons.

88. (New) The method of Claims 71 or 72, wherein contacting one or more cells of said live population of cells with said beam of radiation comprises contacting at least 5,000, 10,000, 20,000, 50,000, 70,000, 100,000, or 150,000 cells per minute with said beam of radiation.

89. (New) The method of Claims 71, wherein contacting one or more cells of said live population of cells with said beam of radiation further comprises the steps of:

pulsing said energy source; and

steering the pulsed beam of radiation from said energy source to multiple spots within said container holding said live population of cells.

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90. (New) The method of Claim 89, wherein said pulsed beam of radiation is wider than any individual cell and is directed to the general area of said live population of cells.

91. (New) A method of rapidly introducing exogenous molecules into a live population of cells, comprising the steps of:

providing a live population of cells in the presence of exogenous molecules;

providing an energy source configured to deliver a beam of radiation;

positioning a container holding said live population of cells within the focus of said beam of radiation; and

contacting one or more cells of said live population of cells with said beam of radiation, wherein said beam of radiation delivers at most $2 \mu\text{J}/\mu\text{m}^2$;

wherein one or more cells of said live population of cells is transiently permeabilized to allow said exogenous molecules to enter.

92. (New) The method of Claim 91, wherein said beam of radiation delivers a value selected from the group of at most 1.5, 1, 0.7, 0.5, 0.3, 0.2, 0.1, 0.05, 0.02, 0.01 or $0.005 \mu\text{J}/\mu\text{m}^2$.

93. (New) The method of Claims 91, wherein said beam of radiation is delivered through a lens with a numerical aperture of at most 0.5.

94. (New) The method of Claim 91, wherein said lens has a numerical aperture selected from the group of at most 0.4, 0.3, or 0.25.

95. (New) The method of Claims 93 or 94, wherein said lens has an F-theta flat-field correction.

96. (New) The method of Claims 91, wherein contacting said one or more cells of said live population of cells with said beam of radiation comprises:

pulsing said energy source; and

steering the pulsed beam of radiation from said energy source to multiple spots within said container holding said live population of cells.

97. (New) The method of Claim 96, wherein said pulsed beam of radiation is wider than any individual cell and is directed to the general area of said live population of cells.

98. (New) The method of Claims 91 or 92, wherein positioning said container holding said live population of cells within said focus of said beam of radiation comprises:

illuminating a field-of-view containing said live population of cells;

analyzing an image of said live population of cells within said field-of-view; and
adjusting the distance between said population of cells and said lens.

99. (New) The method of Claims 91 or 92, wherein the viability of the transiently permeabilized cells is more than 50%, 60%, 70%, 80%, 90%, 95%, or 98% after the method is performed.

100. (New) The method of Claims 93 or 94, wherein the lens comprises a working distance of at least 5, 7, or 10 mm.

101. (New) The method of Claims 91 or 92, wherein contacting one or more cells of said live population of cells with said beam of radiation comprises contacting at least 5,000, 10,000, 20,000, 50,000, 70,000, 100,000, or 150,000 cells per minute with said beam of radiation.

102. (New) The method of Claims 91 or 92, wherein said exogenous molecules comprises a nucleic acid, a polypeptide, a carbohydrate, a lipid or a small molecule.

103. (New) A method of rapidly introducing exogenous molecules into a live population of cells, comprising the steps of:

providing a live population of cells in the presence of exogenous molecules;

providing an energy source configured to deliver a beam of radiation;

positioning a container holding said live population of cells within the focus of said beam of radiation; and

contacting one or more cells of said live population of cells with said beam of radiation, wherein said beam of radiation is delivered through a lens with a numerical aperture of at most 0.5;

wherein one or more cells of said live population of cells is transiently permeabilized to allow said exogenous molecules to enter.

104. (New) The method of Claim 103, wherein said lens has a numerical aperture selected from the group of at most 0.4, 0.3, or 0.25.

105. (New) The method of Claims 103 or 104, wherein positioning said container holding said live population of cells within said focus of said beam of radiation further comprises the steps of:

illuminating a field-of-view containing said live population of cells;

analyzing an image of said live population of cells within said field-of-view; and
adjusting the distance between said population of cells and said lens.

106. (New) The method of Claims 103, wherein contacting said live population of cells with said beam of radiation comprises:

pulsing said energy source; and

steering the pulsed beam of radiation from said energy source to multiple spots within said live population of cells.

107. (New) The method of Claim 106, wherein said pulsed beam of radiation is wider than any individual cell and is directed to the general area of said live population of cells.

108. (New) The method of Claims 103 or 104, wherein contacting one or more cells of said live population of cells with said beam of radiation comprises contacting at least 5,000, 10,000, 20,000, 50,000, 70,000, 100,000, or 150,000 cells per minute with said beam of radiation.

109. (New) The method of Claims 103 or 104, wherein said lens has an F-theta flat-field correction.

110. (New) The method of Claims 103 or 104, wherein said lens comprises a working distance of at least 5, 7, or 10 mm.

111. (New) The method of Claims 103 or 104, wherein the viability of the transiently permeabilized cells is more than 50%, 60%, 70%, 80%, 90%, 95%, or 98% after the method is performed.

112. (New) The method of Claims 103 or 104, wherein contacting said live population of cells with said beam of radiation comprises illuminating said live population of cells with light selected from the group consisting of visible, ultraviolet and infrared wavelengths.

113. (New) The method of Claims 103 or 104, wherein said exogenous molecules comprises a nucleic acid, a polypeptide, a carbohydrate, a lipid or a small molecule.

114. (New) A method of rapidly introducing an exogenous molecule into a live cell, comprising:

providing a live cell in the presence of said exogenous molecule;

providing an energy source configured to deliver a beam of radiation; and

contacting said live cell with said beam of radiation, wherein said live cell is transiently permeabilized to allow said exogenous molecule to enter; and

wherein (a) said beam of radiation has a diameter of at least 2 μm at the point of contact with said live cell, (b) said beam of radiation delivers at most 2 $\mu\text{J}/\mu\text{m}^2$, (c) said beam of radiation is delivered through a lens with a numerical aperture of at most 0.5, or (d) two or more of (a), (b) or (c).

115. (New) The method of Claim 114, wherein said beam of radiation has a diameter of at least 2 μm at the point of contact with said live cell.

116. (New) The method of Claim 115, wherein said diameter of said beam of radiation is at least 5, 7, 10, 15, 20, 25, or 30 μm at the point of contact with said live cell.

117. (New) The method of Claim 114, wherein said beam of radiation delivers at most 2 $\mu\text{J}/\mu\text{m}^2$.

118. (New) The method of Claim 117, wherein said beam of radiation delivers at most 1.5, 1, 0.7, 0.5, 0.3, 0.2, 0.1, 0.05, 0.02, 0.01 or 0.005 $\mu\text{J}/\mu\text{m}^2$.

119. (New) The method of Claim 114, wherein said beam of radiation is delivered through a lens with a numerical aperture of at most 0.5.

120. (New) The method of Claim 119, wherein said lens has a numerical aperture of at most 0.4, 0.3, or 0.25.

121. (New) The method of Claim 114, wherein said beam of radiation is wider than the cell.

122. (New) The method of Claim 114, wherein said beam of radiation is directed to the general area of said cell.